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Sputum or blood eosinophil association with clinical measures of COPD severity in the SPIROMICS cohort

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Contributors

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Declaration of interests

NEA, ERB, REK, WO'N NP and XL report nothing to disclose.

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Abstract

Background—Eosinophils in blood and sputum in chronic obstructive pulmonary disease (COPD) have been associated with more frequent exacerbations, lower lung function, and corticosteroid responsiveness. We hypothesized increased eosinophils are associated with a severe COPD phenotype, including exacerbation frequency, and tested whether blood eosinophils reliably predict sputum eosinophils.

Methods—Comprehensive baseline data on SPIROMICS subjects, recruited for a range of COPD severity for smokers with 20 pack year history, included demographics, questionnaires, clinical assessments, quantitative computed tomography (QCT), blood and induced sputum.

Findings—Significantly, stratification by mean sputum eosinophils 1.25% (N=827) was associated with reduced FEV₁ % predicted (differences: 10% pre-bronchodilator, 4.7% post-bronchodilator), QCT density measures for emphysema and air trapping, and exacerbations treated with corticosteroids (p=0.002). In contrast, stratification by mean blood eosinophils 200/μL (N=2499) showed that FEV₁ % predicted was significant between low and high blood subgroups, but less than observed between sputum subgroups (blood eosinophil group differences: 4.2% pre-bronchodilator, 2.7% post-bronchodilator), slightly increased airway wall thickness (0.02 mm, p=0.032), greater symptoms (p=0.037), and wheezing (p=0.018), but no evidence of association with COPD exacerbations or other indices of severity. Blood eosinophils showed weak although significant association with sputum eosinophils (ROC AUC=0.64, p<0.001), but with a high false discovery rate (72%). Elevated sputum eosinophils, with or without blood eosinophils, were associated with lower lung function. Elevated blood eosinophils only in combination with elevated sputum eosinophils were associated with COPD exacerbations.

Interpretation—Stratification of SPIROMICS subjects by blood eosinophils alone showed minimal clinical differences and no association with exacerbations, whereas stratification by sputum eosinophils was associated with larger phenotypic differences and COPD exacerbations. Importantly, increased blood eosinophils did not reliably predict airway eosinophils in induced sputum.

Keywords

COPD severity; airway eosinophilia; emphysema; hyperinflation; air-trapping

INTRODUCTION

Airways inflammation in chronic obstructive pulmonary disease (COPD) is thought to be characterized by increased neutrophils,¹ macrophages,² proteases, IL-6, IL-8, and Th1 cytokines³ while airways inflammation in asthma is traditionally characterized by increased eosinophils, and Th2 cytokines.⁴ However, reports challenge these presumptive differences between asthma and COPD. The ECLIPSE study reported that in COPD, sputum neutrophils are weakly associated with lung function and health status, and not associated with exacerbations, emphysema or systemic inflammation.¹ ECLIPSE also reported a mean 1.3%

sputum eosinophil level in 359 subjects with COPD,¹ but did not observe blood eosinophil associations with radiologic measure of emphysema or with COPD exacerbations and hospitalizations. ECLIPSE reported 2% (150/ μ L) blood eosinophils associated with evidence of higher FEV1, lower St. Georges Respiratory Questionnaire (SGRQ) and modified Medical Research Council scores.⁵ Other COPD studies have reported increased eosinophils associated with exacerbations and greater hyperinflation on QCT,^{6,7} suggesting Th2 inflammation may contribute to disease progression. Moreover, increased epithelial Th2 signature gene expression has been associated in two COPD cohorts with more severe airflow obstruction.⁸ Eosinophils may represent a potential biomarker in COPD since eosinophilia is related to corticosteroid responsiveness.^{1,9–11} In a phase II clinical trial, anti-IL-5 receptor therapy reduced the COPD exacerbation rate in a subgroup of patients with elevated blood and sputum eosinophilia.¹²

Determination of disease severity in COPD is complex and involves more than lung function assessments; additional clinical characteristics have been incorporated in successive revisions of the GOLD severity stages.¹³ Current classification includes lung function, symptom scores and exacerbation frequency. Thus, severity of COPD is dependent on multiple characteristics; eosinophilic inflammation may contribute.

Reports suggest blood eosinophil counts may represent a useful surrogate measure of airway eosinophils in COPD,^{11,14} although blood eosinophils appear to correlate poorly with sputum eosinophils in asthma,^{15,16} and do not distinguish between asthma-dominant, COPD-dominant or asthma/COPD overlap populations.¹⁷ However, larger studies of comprehensively phenotyped COPD patients often lack robust sputum eosinophil data.^{18–21} Thus it is uncertain whether peripheral eosinophils accurately predict airway eosinophils.

We investigated the hypotheses that blood and sputum eosinophils in subjects with a history of tobacco use were associated with a more severe COPD phenotype identified by diminished lung function, QCT measurements of emphysema or air-trapping, clinical COPD characteristics, and exacerbations. We also investigated relationships of blood and sputum eosinophils to determine whether blood eosinophils reliably predicted sputum eosinophils. These hypotheses were evaluated in the comprehensively characterized SPIROMICS cohort.²² A portion of these studies were presented as an abstract at the 2016 American Thoracic Society meeting.²³

METHODS

Subjects

Subjects with current or former history of tobacco use (≥ 20 pack-year), recruited to include specific groups of smokers with preserved lung function (31%), GOLD stages 1 and 2 (41%), and GOLD stages 3 and 4 (21%) and a control group of nonsmokers, age 40–80 (N=2737), were enrolled in the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) at six clinical sites and additional subsites.²² COPD was defined in long-term smokers as a post-bronchodilator FEV1/FVC ratio <0.7 . Subjects underwent extensive baseline phenotypic characterization including lung function assessment pre- and post-bronchodilator with albuterol and ipratropium, CT total lung

capacity and residual volume (TLC and RV) using QCT indicators for emphysema (% voxels less than -950 Hounsfield Units [HU]) at TLC, and air-trapping (% voxels less than -856 HU) at RV, airway metrics (VIDA Diagnostics, Iowa)²⁴ and parametric response mapping (PRM) for functional small airways disease (fSAD),²⁵ collection of blood (for DNA, RNA, plasma, sera, IgE and complete blood cell counts [CBC]), urine, 6 minute walk distance, GOLD stage, BODE index, COPD Assessment Score (CAT), St. Georges Respiratory Questionnaire (SGRQ), and administered questionnaires for medical history, exacerbations (retrospective from past year), hospitalizations, respiratory exposures, and medications.²² In a subset (see online supplement Figure S1 detailing subset of SPIROMICS with induced sputum cell counts), induced sputum was performed. Subjects with a primary asthma diagnosis were excluded, but all subjects were asked if they had ever had a health care professional say that they had asthma ("prior asthma label"). In the N=2499 enrolled ever smokers, the mean \pm standard deviation for blood eosinophil count was $200 \pm 240/\mu\text{L}$ (median=190/ μL ; full range 0–8300/ μL); a higher eosinophil cutpoint of 300/ μL was also examined. See supplement for additional information on the selection of stratification cutpoints, reproducibility, and other details.

Sputum Induction and Processing

SPIROMICS subjects with post-bronchodilator FEV₁ % predicted $\geq 35\%$ were eligible for sputum induction with saline solutions, nebulized for three 7 min intervals each. Expecterated sputum samples were processed as described in detail in the supplement. Cytospin slides were read by the central reading center. Slides were available for 1001 subjects. Differential counts (500–600 total cells) which had ≥ 100 leukocytes total (N=179) or $\geq 80\%$ squamous (N=11) were excluded as unacceptable. The mean sputum eosinophil % (\pm standard deviation) for the subgroup with acceptable sputum (N=827) was $1.25 \pm 4.25\%$ (median=0.3%; full range 0–75%); a higher cutpoint of 2% for sputum eosinophils was also examined. (See supplement on subject distribution with induced sputum.)

Statistical Analyses

Subjects were stratified by eosinophil mean blood counts or mean sputum %. Demographic and biomarker data are presented as means \pm standard deviations, or medians (25%–75% interquartile range) for continuous variables, and as % positive for categorical variables. Measures not meeting Kolmogorov-Smirnov test for normal distribution, were transformed by log, or square root values. Continuous variables were tested by parametric or non-parametric tests (see supplement; SAS 9.2, or Sigmastat 12.5). Categorical variables were analyzed using Chi-square or Fisher Exact tests. Correlations were examined by Pearson Correlation test or linear regression. Receiver Operating Curve (ROC) analysis was performed for blood eosinophil prediction of sputum eosinophils. The False Discovery Rate (= False Positives / False Positives + True Positives) was examined.²⁶ Classification tree analysis examining sputum and blood eosinophils to model exacerbations was performed using R part routines in R software package. Variables with a p value <0.05 were accepted as significant.²⁷

RESULTS

Subject Demographics

Demographic characteristics of SPIROMICS subjects stratified by mean blood eosinophils ($< 200/\mu\text{L}$) and by mean sputum eosinophils ($< 1.25\%$) are shown in Table 1. For those subjects with low compared to high blood eosinophils, age, gender, race, BMI, cigarette smoking pack-years, % current smoker, and % use of inhaled corticosteroids (ICS) statistically differed, although differences between the groups were small ($<10\%$). The proportion of current smokers was less in the $200/\mu\text{L}$ eosinophil group, but the number of cigarettes smoked each day was the same. Medications (supplement Table S1) did not differ, except for ICS treatment, higher in the $200/\mu\text{L}$ eosinophil group (Table 1). Total serum IgE levels (range 5.5–1660) and sputum eosinophils % differed in blood eosinophil groups, but those with a “prior asthma label” or childhood asthma did not differ (Table 1). Median sputum eosinophil % in low and high blood eosinophil groups differed ($p<0.001$), but were lower than the overall sputum eosinophil mean of 1.25% .

In subjects stratified by mean sputum eosinophils at 1.25% , age, gender, race, BMI, smoking pack-years, cigarettes/day, and % current smokers did not differ (Table 1). Differential counts for leukocytes in sputum eosinophil groups are presented in supplement Table S2). Medication use in the sputum cohort did not differ, except increased use of ICS, and inhaled or nebulized bronchodilators in the 1.25% sputum eosinophils group (supplement Table S1). Higher sputum eosinophils, unlike higher blood eosinophils, did not have significantly increased IgE levels compared to low sputum eosinophil group. Nevertheless, IgE levels in low and high sputum eosinophil groups were similar to IgE levels in low and high blood eosinophil groups, respectively. In addition, the sputum eosinophil 1.25% group had elevated blood eosinophils ($230/\mu\text{L}$, $p<0.001$) and a greater proportion of subjects reporting a “prior asthma label” ($p=0.003$). Similar results for blood or sputum eosinophil stratification were obtained with higher cutpoints ($300/\text{mL}$ blood eosinophils levels, or 2% sputum eosinophils, respectively; supplement Table S3).

Spirometry

Dividing by blood eosinophils, the $200/\mu\text{L}$ high eosinophil group had marginally lower values for pre-bronchodilator FEV_1 % predicted (4.2%), and no difference post-bronchodilator (0.6%), compared to the $<200/\mu\text{L}$ low eosinophil group (Table 2). The sputum eosinophils 1.25% group had greater differences between pre bronchodilator and post-bronchodilator FEV_1 % predicted (10 and 5.7% , respectively), compared to $<1.25\%$ group. Due to safety exclusion of subjects with post-bronchodilator FEV_1 % predicted $<35\%$ from sputum induction, fewer GOLD Stages 3 and 4 subjects were included in the sputum cohort. However, baseline and post-bronchodilator FEV_1 % predicted showed larger differences between low and high sputum eosinophil groups than observed between low and high blood eosinophil groups. Reversibility of baseline FEV_1 % predicted, was larger in the elevated sputum eosinophil group ($p<0.001$), but did not differ for the blood eosinophil groups. Similar observations were found between subgroups stratified by $300/\mu\text{L}$ blood and 2% sputum eosinophil cutpoints (supplement Table S4).

Imaging

Indices of emphysema and air trapping at TLC and RV²⁴, respectively, did not differ between blood eosinophil groups (Table 3). In contrast, significantly higher emphysema indices (% voxels <−950 HU) in left upper and lower lobes, and right upper lobe were observed in sputum eosinophil 1·25%. In addition, air trapping (% voxels <−856 HU) and functional small airways disease assessed by parametric response mapping (PRM fSAD,²⁵) were higher in subjects with 1·25% sputum eosinophils.

There was a small, 0·02 mm increase in average airway wall thickness at RB1 (prespecified pathway in apical segment of right upper lobe) for elevated blood eosinophils, but not for elevated sputum eosinophils. Neither blood nor sputum stratification showed any difference in airway tapering (an index of bronchiectasis).

The higher 300/μL blood eosinophil cutpoint did not alter density measures for emphysema or air trapping, but reduced significance for RB1 airway wall thickness. The higher 2% sputum eosinophil cutpoint maintained significance for both emphysema and air trapping indices (supplement Table S5).

Clinical Characteristics

Among subjects with 1·25% sputum eosinophils, there were fewer GOLD Stage 0 and increased GOLD Stage 2 subjects compared to subjects with <1·25% sputum eosinophils ($p=0\cdot0006$, Table 4). The 6 min walk distance, BODE Index and COPD Assessment Score did not differ for either blood or sputum eosinophil stratifications. The blood eosinophil 200/μL group showed significantly higher frequency of self-reported wheezing (Table 4). St. George Respiratory Questionnaire (SGRQ) symptom score was also higher in the 200/μL blood eosinophil subgroup; both SGRQ total and symptom scores were significantly higher in the 1·25% sputum eosinophils subgroup.

The higher 300/μL blood eosinophils cutpoint showed a significant difference for GOLD Stages (supplement Table S6). The higher 2% sputum eosinophil cutpoint maintained significance for GOLD Stages, SGRQ total and symptoms scores, and became significant for BODE Index, SGRQ Impact and self-reported wheezing.

Exacerbations

Blood eosinophils 200/μL and sputum eosinophils 1·25% were tested for association with exacerbations (Table 5). Elevated blood eosinophils were not associated with any of the different categories of reported exacerbations. In contrast, elevated sputum eosinophils were associated with increased proportions of subjects with exacerbations requiring corticosteroids, exacerbations requiring treatment with any drug, and severe exacerbations requiring emergency department visit. The higher 300/μL blood eosinophil cutpoint did not show any association with exacerbations, but the higher 2% sputum eosinophil cutpoint demonstrated significance for all categories of reported COPD exacerbations (supplement Table S7). Tree classification of sputum and blood eosinophil association with exacerbations selected sputum eosinophils before blood eosinophils and showed similar cutpoints, sequentially < or 1·9% for sputum eosinophils and < or 176/μL for blood eosinophils,

supporting the 2% and 200/ μ L cutpoints investigated for sputum and blood eosinophils in this study (supplement Figure S2). Sputum eosinophils $>1.9\%$ identified a subgroup of subjects with exacerbations (27 of 119 or 23%). In contrast, those with $<1.9\%$ sputum eosinophils showed a lower proportion of subjects with exacerbation (65 of 692 or 9%).

Blood Eosinophil Prediction of Sputum Eosinophils

ROC analyses demonstrated a relatively weak, although significant relationship for blood eosinophils to predict sputum eosinophils $\geq 1.25\%$ (Figure 1, AUC=0.63, $p<0.0001$); ROC to predict $\geq 2\%$ sputum eosinophils was similar (supplement Figure S3). Highest sensitivity and specificity for predicting sputum eosinophils $\geq 1.25\%$ and $\geq 2\%$ were found at 150/ μ L and 250/ μ L blood eosinophils, respectively, with equivalent, significant AUCs observed at adjacent cutpoints (supplement Table S8). Nevertheless, both associations had very large false discovery rates; 72% for blood eosinophils $\geq 150/\mu$ L to predict sputum eosinophils $\geq 1.25\%$ (false negative rate of 22%), and 74% for blood eosinophils $\geq 250/\mu$ L to predict sputum eosinophils $\geq 2\%$ (false negative rate of 50%).

Combined Blood and Sputum Eosinophil Phenotypes

The correlation between sputum eosinophils and blood eosinophils was poor, but significant (Figure 2; correlation coefficient $r=0.178$, $p<0.001$). However, numerous subjects (42%) had discordant blood and sputum eosinophil levels; either high in blood or in sputum, but not in the other compartment. Lung function and reported exacerbations for subjects in the two discordant quadrants and two concordant quadrants were compared (Table 6). High sputum eosinophil groups, without or with high blood eosinophils, had the lowest lung function. Lung function for the high blood eosinophil group without high sputum eosinophils did not differ from the group with low eosinophils in both blood and sputum. However, high blood eosinophil groups only in combination with elevated sputum eosinophils had more COPD exacerbations; exacerbations treated with corticosteroids ($p=0.006$) or severe ($p=0.013$) were significant.

Additional Stratifications for Blood and Sputum Eosinophil Subgroups

Subjects were stratified by “prior asthma label” or ICS use and examined for interaction with high eosinophils (blood eosinophils $\geq 200/\text{mL}$ or sputum eosinophils $\geq 1.25\%$) on lung function and exacerbations. No interactions were significant (supplement Tables S9, and S10). Subjects who did not have acceptable sputum slides for various reasons ($N=1498$) were stratified by blood eosinophil counts to determine whether these subjects represented a phenotype with different characteristics (supplement Table S11). There was slightly higher proportion of subjects using ICS, lower lung function and increased proportions of GOLD Stage 3 and 4 subjects as would be expected in these groups which contained subjects with lower lung function and therefore ineligible for sputum induction, but otherwise resembled the larger cohort of smokers.

Another stratification examined whether blood eosinophil groups $<$ or $\geq 200/\mu$ L showed differences when restricted to just those subjects in the sputum cohort (supplement Table S12). The sputum cohort divided into blood eosinophil subgroups had slightly greater proportion of current smokers, less ICS use and slightly better lung function, but did not

show the same radiologic, clinical or exacerbation differences observed for sputum eosinophil stratification.

We examined whether associations with worse lung function and quality of life, and greater exacerbations, emphysema, and air trapping in the high sputum eosinophil group were due to elevated sputum neutrophils in addition to high sputum eosinophils. There was no difference in mean sputum neutrophil % between high and low sputum eosinophil groups (Table S2). Stratification of the sputum cohort into 4 groups based on $< \text{or } 1\text{--}25\%$ eosinophils + $< \text{or } > 68\%$ neutrophils (mean \pm std deviation for sputum neutrophils: $68\% \pm 21\%$), confirmed differences across low and high sputum eosinophil groups but did not show significant post-hoc differences between the high eosinophil + high neutrophil and high eosinophil + low neutrophil subgroups (Supplement additional results, Table S13).

DISCUSSION

The results of this study from the SPIROMICS cohort, a smoking cohort that includes a spectrum of COPD severity defined by GOLD stages, confirms that elevated sputum eosinophils, but not blood eosinophils alone, identify a subset of COPD subjects with more severe airflow obstruction, worse quality of life, greater emphysema and air trapping, and exacerbations. Using sputum eosinophil stratification at either the mean, $1\text{--}25\%$, or 2% , we found significant associations with COPD exacerbations, including severe and those requiring corticosteroid therapy. In addition, significant associations were found for lower lung function, baseline and post-bronchodilation including increased bronchodilator reversibility; respiratory symptoms; emphysema and air trapping by QCT; and COPD severity by GOLD Stage. In contrast, blood eosinophils alone, at $200/\mu\text{L}$, or the even higher cutpoint at $300/\mu\text{L}$, showed no association with COPD exacerbations, and associations with other phenotypic markers were smaller or non-significant. Although there was an increase in SGRQ symptom scores for higher eosinophils in both blood and sputum compartments, and wheeze (found only for blood, possibly due to the larger N for that group) the differences between low and high eosinophil groups for these variables were greater in the sputum group. In addition, there was no difference in CAT scores for either blood or sputum, which tends to diminish the validity for this observation. Importantly, although the relationship between blood and sputum eosinophilia was statistically significant, blood eosinophils did not reliably predict sputum eosinophils, showing a 72–74% false-discovery rate and a 50% false negative rate for sputum eosinophils 2% . Lung function data stratified by high and low sputum and blood eosinophils showed no relationship with high blood eosinophils unless combined with high sputum eosinophils, while high sputum eosinophils even in the absence of blood eosinophils was associated with lower lung function. However, COPD subjects with both high sputum and blood eosinophils exhibited both decreased lung function and more frequent exacerbations. These findings among current and former smokers in a large multicenter cohort with a specified range of COPD severity have important implications for proposed use of blood eosinophils alone as a predictive biomarker to guide individualized COPD therapies.

Our results extend observations from previous studies in COPD cohorts, including ECLIPSE, which focused primarily on neutrophilic airways inflammation,¹ and, though

reporting eosinophil presence,⁵ did not address association of eosinophilia with indices of COPD severity.^{4,5} The importance of our findings and of Th2 inflammation in COPD are emphasized by the recent report of Th2 gene expression overlap in airway epithelial samples from asthma and COPD cohorts,⁸ and by shared clinical and biologic characteristics between asthma and COPD reported in several recent studies.^{5,7,17,28–29} However, differences are noted between SPIROMICS and other COPD cohorts. COPD gene enrolled a larger cohort (N=10,000) which was slightly older (minimum 45 yr) and had a lower smoking history (>10 pack year), but phenotyping with induced sputum was not performed.³⁰ Sputum was also unavailable in Copenhagen General Population Study,²⁰ WISDOM,²¹ INSPIRE, and TRISTAN.³¹ Although these studies report exacerbations correlating with blood eosinophils, it is important to note that the entry requirements included past history of COPD exacerbations which can impact the results since a past history of exacerbation is the most important factor predicting future exacerbations.¹⁸

In addition emphasis on persistent Th2 inflammation in COPD³², has focused on eosinophils as predictors of exacerbations. Bafadhel and colleagues reported a cluster analysis using blood and sputum biomarkers; peripheral blood eosinophils predicted sputum eosinophil-associated exacerbations of COPD.⁶ Sputum airway and peripheral blood eosinophils have been used to direct corticosteroid treatment and reduce occurrence of COPD exacerbations.^{9–11} The ECLIPSE study reported that 1483 subjects if stratified by blood eosinophils did not differ for COPD exacerbation rate in the previous year.⁵ We confirm that higher blood eosinophils are not associated with COPD exacerbations except combined with elevated sputum eosinophils or with other characteristics such as a previous history of exacerbation.¹⁸ However, in SPIROMICS higher sputum eosinophils alone are associated with exacerbations even in mild to moderate COPD.

Eosinophil levels have been suggested to indicate response to corticosteroids, anti-IL5, or anti-IL5 receptor therapy.^{9–12,33} In a retrospective analysis of two COPD exacerbation studies with long-acting beta-agonists and inhaled corticosteroids, Pascoe showed that subjects with higher blood eosinophils had greater reduction in COPD exacerbations.³⁴ These observations suggest eosinophils may be important in development or potential biomarker of some COPD exacerbations. However, two factors may influence previous observations correlating elevated blood eosinophil groups with greater COPD exacerbations; selection criteria requiring recent exacerbation and perhaps lower lung function are both related to future COPD exacerbations and may be surrogate markers of increased sputum eosinophils. Elevated blood eosinophils, if also combined with elevated sputum eosinophils, associate with COPD exacerbations, as shown for the SPIROMICS cohort in this report. However, blood eosinophils alone were not associated with exacerbations, even when combined with “prior asthma label”. This latter observation contrasts with the association of blood eosinophils $\geq 275/\mu\text{L}$ with all cause mortality in 662 subjects, but that study found no change after exclusion of subjects with asthma.³⁵ Blood eosinophils in our study were associated with COPD exacerbations only in the context of higher sputum eosinophils.

We also examined other characteristics in the blood and sputum eosinophil subgroups of SPIROMICS subjects that might suggest overlap with asthma: bronchodilator reversibility, IgE levels, and childhood asthma. Blood eosinophils at two different cutpoints (either 200

or $300/\mu\text{L}$) did not have higher levels of acute bronchodilator reversibility, while elevated sputum eosinophils showed greater reversibility. IgE levels were significantly higher in the elevated blood eosinophil group, although not in sputum, but IgE levels in SPIROMICS blood and sputum eosinophil subgroups were well below median (91 IU/ml) and high IgE cutpoint (173 IU/ml) reported in a recent study of asthma-COPD overlap syndrome.³⁶ Only 2% sputum eosinophils showed increased report of childhood asthma, which represent a small subgroup (12.5%).

There was greater use of prescribed inhaled corticosteroids in both high blood and sputum eosinophil groups. This was observed despite an expected reduction in eosinophils with corticosteroid therapy. Use of corticosteroids in the higher eosinophil groups potentially reflects individuals more likely to have had exacerbations, consistent with GOLD guideline recommendations for corticosteroids in COPD patients with frequent exacerbations.¹³ Limitations of this report include somewhat milder COPD in the group who were able to successfully perform induced sputum. For safety reasons, SPIROMICS subjects with post-bronchodilator FEV1 % predicted $<35\%$ did not have sputum induction, limiting the sputum subgroup to GOLD Stages 0–3. Although our cohorts (both for blood and sputum eosinophil analyses) included smoking subjects (>40 pack years) with preserved lung function, these subjects were included because they have been shown to have greater symptoms, exacerbations, activity limitations and radiologic evidence of airway disease.³⁷ These findings are consistent with early COPD in this subgroup.³⁷ Although SPIROMICS exacerbations data was retrospective, validity of retrospective data for future risk of COPD exacerbation has been shown in the ECLIPSE where self-reported exacerbation from the previous year had predicted exacerbations during the first year of follow up, more accurately than all other variables examined.¹⁸ An additional limitation, at least in clinical settings, is the difficulty in performing accurate sputum analysis. Even in the SPIROMICS network with centralized training for sputum induction and processing there were still reasons preventing sputum analysis on all eligible subjects as indicated in the supplemental methods. However analysis of those who did not have sputum analysis, stratified by blood eosinophils did not differ substantially from the larger cohort.

Of interest, longitudinal follow-up of the SPIROMICS cohort may be used to confirm the observations of Hoppers and colleagues that peripheral eosinophils are associated with all cause mortality over a period of 30 years.³⁵ Alternatively, the differences in decline of lung function associated with blood eosinophils $<$ or $\geq 2\%$ observed in the much smaller study over 9 years by Rogliani and colleagues should be examined in the larger SPIROMICS cohort longitudinally.³⁸ In summary, using the larger and comprehensive phenotypic characterization of the SPIROMICS cohort, we show that stratification by elevated sputum eosinophil inflammation identified a subgroup with more severe COPD, having decreased lung function, greater emphysema and air trapping, and greater COPD exacerbations. Peripheral blood eosinophils identified a subgroup with decreased lung function without other indices of more severe COPD, specifically exacerbations unless examined on the background of elevated sputum eosinophils. Moreover, blood eosinophils did not accurately predict sputum eosinophils. These observations confirm the importance of assessing eosinophils in the airways. In the future, it will be important to follow these subjects with

higher sputum eosinophilia longitudinally to determine whether long term effects on the progression of COPD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in Context

Evidence before this study

A PubMed search for original research reports containing information on eosinophils, sputum, blood and COPD through May 2017 yielded 154 articles, of which 32 were reviews. Addition of either “severity” or “exacerbation” reduced publication numbers to 33 (7 reviews) or 35 (1 review), respectively. However, many of these reports have further limitations, either lacking sputum or blood eosinophil data for comparison, not specifically focused on severity of COPD including exacerbations, or containing small numbers of subjects (<100/group) which limit the power to make conclusions for broader COPD populations. Generally eosinophils in COPD have been linked to more frequent exacerbations and responsiveness to corticosteroid therapy, suggesting more severe disease. Often studies are primarily in populations who have met selection criteria for clinical trials that include the presence of COPD exacerbations. Thus, comparison of blood eosinophils and sputum eosinophils for association with a more severe COPD phenotype has not been well studied in a general smoking population with a broad range of COPD severity, nor has possible substitution of blood eosinophils as a biomarker for sputum eosinophils in COPD populations been carefully examined.

Added value of this study

This study demonstrates that in a large, comprehensively characterized smoking cohort with a broad range of COPD severity, elevated sputum eosinophils, but not blood eosinophils alone, had significant associations with multiple measures of COPD severity, including exacerbations, increased emphysema and air trapping, St. George Respiratory Questionnaire scores and GOLD spirometric stage. Blood eosinophils demonstrated weak association with sputum eosinophils and as a single biomarker had few significant associations with COPD severity and exacerbations. However, this study does demonstrate that elevated blood eosinophils in combination with elevated sputum eosinophils show associations with COPD exacerbations and severity.

Implications of all the available evidence

Increased sputum eosinophils from subjects with a broad range of COPD severity identify those more likely to have severe disease and exacerbations. Blood eosinophils as a single biomarker do not accurately predict sputum eosinophils, and do not show any association with disease severity or exacerbations unless observed in the background of increased sputum eosinophils. The findings from this study will be important in the design of therapeutic trials which target eosinophilic inflammation in COPD. This article has an online data supplement.

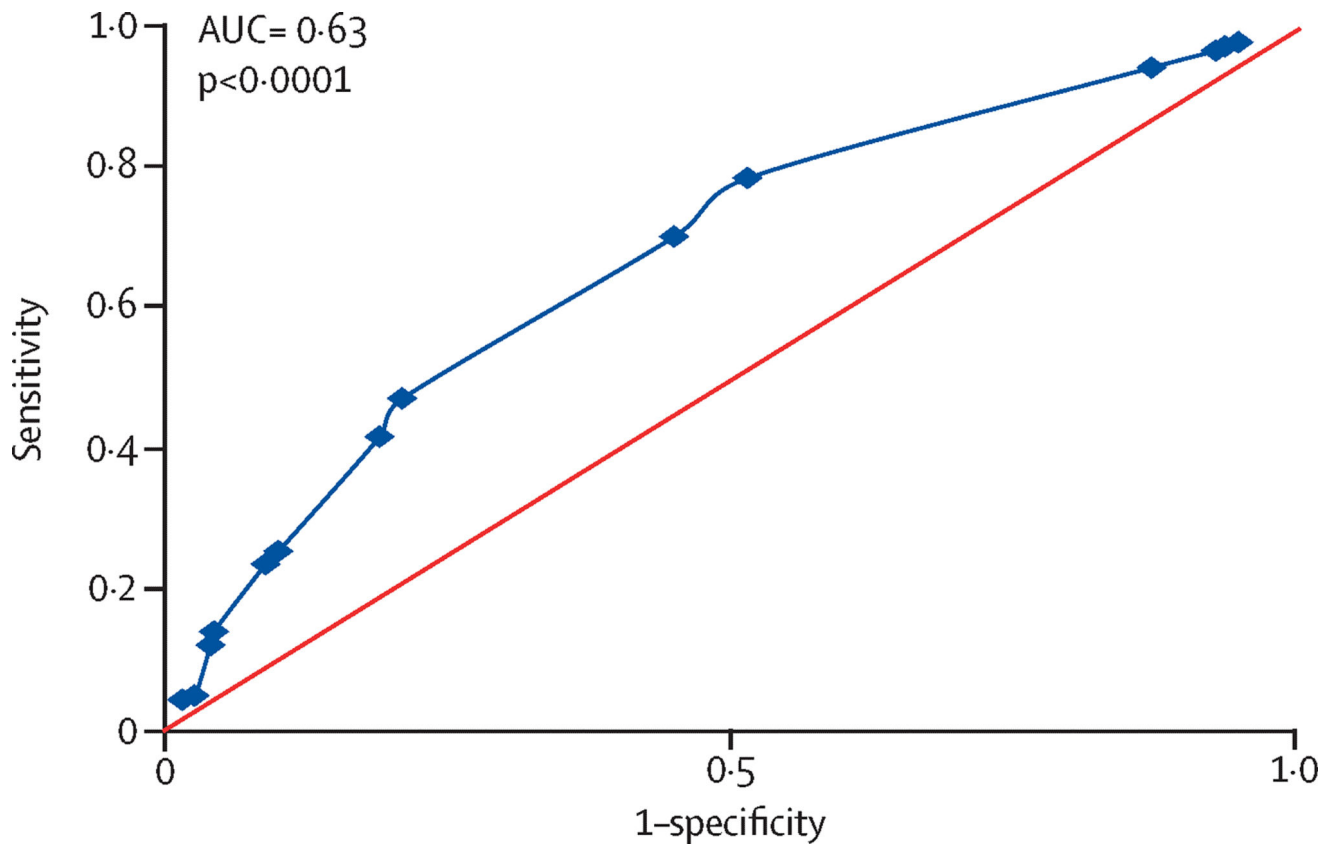


Figure 1.

ROC analysis for blood eosinophil prediction of sputum eosinophil. Blood eosinophils at cutpoints from 50/ μ L (highest sensitivity) to 500/ μ L (lowest sensitivity) were examined for correct prediction of sputum eosinophils \leq 1.25%. Although significant ($p < 0.001$), the area under the curve (AUC) was only 0.63, demonstrating a lack of strength for the prediction. Maximum sensitivity and specificity were observed at a blood eosinophil cutpoint of 150/ μ L.

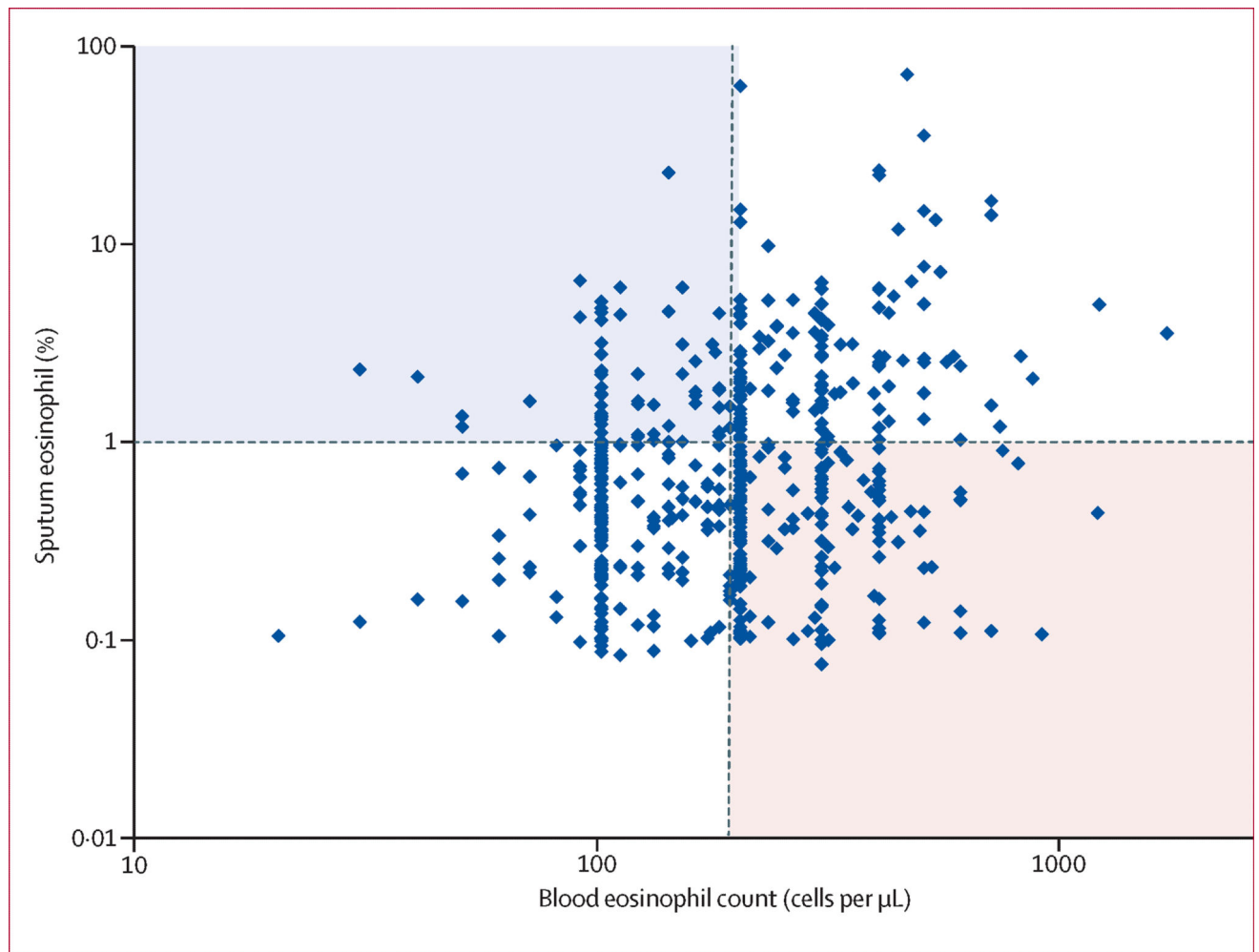


Figure 2.

Distribution of subject's blood eosinophils with respect to sputum eosinophils. Although there is a significant association between blood and sputum eosinophils (EOS) (Pearson correlation coefficient $r=0.178$, $p<0.001$), use of the cutpoint 200/ μL blood eosinophils (vertical red line) to predict sputum eosinophils 1.25% (horizontal red line) will mistakenly identify many subjects with lower sputum eosinophil% (lower right quadrant, pink shade) and miss many subjects with actual sputum eosinophils 1.25% (upper left quadrant, blue shade).

Table 1

Demographics for subjects stratified by mean blood or sputum eosinophils (Eos; cutoff 200/ μ L or 1.25%, respectively).

Variable	Blood Eos<200/ μ L	Blood Eos 200/ μ L	P Value*	Sputum Eos<1.25%	Sputum Eos 1.25%	P Value*
Number	1262	1237		656	171	
Age (yr)	65 (56 – 70)	65 (59 – 71)		65 (57–71)	64 (57–71)	0.87
Male Sex, N (%)	631 (50)	730 (59)		377 (57)	99 (58)	0.99
RACE N (%) Cau/N (%) AA/N (%) other	934 (74)/ 285 (23)/ 43 (3)	1004 (81)/ 173 (14) / 60 (5)		511 (78)/ 110 (17)/ 35 (5)	132 (77)/ 29 (17)/ 10 (6)	0.90
BMI	26.8 (24 – 31)	28.2 (25 – 32)		28.3 (25–32)	28.1 (25–32)	0.92
Smoking (pack-years)	41 (30 – 60)	45 (34 – 60)		43 (32–60)	44 (33–60)	0.70
Cigarettes/day	15 (9 – 20)	15 (8 – 20)	0.97	15 (10–20)	15 (6–20)	0.52
Current Smoker, N (%) positive	522 (42)	451 (37)		293 (45)	70 (41)	0.39
ICS, N (%) positive	404 (32)	470 (38)		169 (26)	66 (39)	
IgE (geometric mean)	34 (14–93)	49 (19–166)		41 (16–104)	56 (15–203)	0.32
Sputum Eos %	0.23 (0.0–0.76)	0.65 (0.12–2.42)				
Blood Eos count/ μ L				150 (100–200)	230 (160–350)	
“Prior asthma”, N (%) positive	249 (20)	255 (21)	0.48	122 (19)	48 (29)	
Childhood asthma, N (%) positive	97 (7.7)	118 (9.5)	0.10	52 (7.9)	20 (11.7)	0.07

* Mann-Whitney rank sum test for continuous variables, results as median (25–75% interquartile range); Chi-square for categorical variables, results as N (% positive response).

Lung function for subjects stratified by mean blood or sputum eosinophils (Eos; 200/ μ L cutoff or 1.25% cutoff, respectively).

Table 2

Variable	Blood Eos<200/ μ L	Blood Eos 200/ μ L	P Value*	Sputum Eos<1.25%	Sputum Eos 1.25%	P Value*
Number	1262	1237		656	171	
<i>Pre-bronchodilator:</i>						
FEV ₁ (L)	1.86 (1.22–2.54)	1.81 (1.16–2.55)	0.38	2.15 (1.57–2.77)	1.83 (1.38–2.32)	
FEV ₁ % predicted	70.5 (46.6 – 88.2)	66.3 (42 – 85.6)		75.7 (59.3–90.2)	65.7 (52–81.3)	
FVC % predicted	87.3 (73–99)	85.5 (72–97)		90.9 (79–100)	87.1 (77–97)	0.06
FEV ₁ /FVC	0.64 (0.49 – 0.73)	0.61 (0.47 – 0.72)		0.66 (0.58 – 0.74)	0.61 (0.52 – 0.69)	
<i>Post-bronchodilator:</i>						
FEV ₁ (L)	2.05 (1.43–2.72)	2.03 (1.39–2.75)	0.62	2.34 (1.78–2.59)	2.11 (1.69–2.59)	
FEV ₁ % predicted	75.9 (53–94)	76.5 (53–92)	0.85	82.9 (68–96)	77.2 (63–88)	
FVC % predicted	92.9 (81–104)	90.5 (79 – 102)		94.5 (85 – 105)	94.2 (86 – 104)	0.84
FEV ₁ /FVC	0.66 (0.5 – 0.76)	0.63 (0.49 – 0.74)		0.68 (0.59 – 0.76)	0.64 (0.55 – 0.72)	
% FEV ₁ reversibility	9.3 (4.2 – 17.7)	9.8 (4.5 – 19)	0.46	8 (3.7 – 15.4)	11.6 (6 – 21.7)	

* Mann-Whitney rank sum test for continuous variables, results as median (25–75% interquartile range).

Table 3

Imaging parameters for subjects stratified by mean blood or sputum eosinophils (Eos; cutoff 200/ μ L, or cutoff 1.25%, respectively).

Variable	Blood Eos<200/ μ L (N=1262)	Blood Eos 200/ μ L (N=1237)	P value*	Sputum Eos<1.25% (N=656)	Sputum Eos 1.25% (N=171)	P value*
<i>DENSITY MEASURES</i>						
TLC Left Upper Lobe %<-950 HU	3.22 (1.11 – 11.84)	3.77 (1.20 – 11.50)	0.66	2.24 (0.89 – 5.74)	2.88 (1.09 – 7.65)	
TLC Right Upper Lobe %<-950 HU	2.78 (0.72 – 12.97)	2.87 (0.73 – 11.96)	0.76	1.73 (0.6 – 5.58)	2.43 (0.9 – 7.24)	
TLC Left Lower Lobe %<-950 HU	2.09 (0.81 – 7.09)	2.42 (0.88 – 7.5)	0.23	1.60 (0.72 – 3.78)	1.98 (0.76 – 5.32)	
RV BOTH Lungs %<-856 HU	17.4 (6.71 – 39.38)	18.91 (7.39 – 40.71)	0.28	12.52 (5.34 – 25.27)	17.20 (8.57 – 33.13)	
PRM functional small airway disease	14 (4 – 33)	15 (4 – 34)	0.21	9 (3 – 22)	13 (6 – 26)	
<i>AIRWAY MEASURES</i>						
AVG WALL THICK apical right upper lobe	1.26 (1.15 – 1.37)	1.28 (1.16 – 1.39)		1.28 (1.18 – 1.38)	1.29 (1.19 – 1.42)	0.08
Taper Ratio - apical right upper lobe	0.038 (–0.01 – 0.086)	0.039 (–0.01 – 0.09)	0.87	0.03 (–0.01 – 0.08)	0.03 (–0.01 – 0.08)	0.93

* Mann-Whitney rank sum test for continuous variables, results as median (25–75% interquartile range).

Table 4

Clinical characteristics for subjects stratified by mean blood or sputum eosinophils (Eos; cutoff 200/ μ L or 1.25%, respectively).

Variable	Blood Eos<200/ μ L (N=1262)	Blood Eos 200/ μ L (N=1237)	P value*	Sputum Eos<1.25% (N=656)	Sputum Eos 1.25% (N=171)	P value*
GOLD Stage 0 N(%) / 1 N(%) / 2 N(%) / 3 N(%) / 4 N(%)	505 (40) / 150 (12) / 323 (26) / 190 (15) / 79 (6)	425 (34) / 153 (12) / 359 (29) / 200 (16) / 86 (7)	0.10	295 (45) / 106 (16) / 200 (31) / 47 (7) / 0 (0)	51 (30) / 31 (18) / 76 (44) / 11 (6) / 1 (1)	
6 Minute Walk Distance (m)	418 (354 – 482)	410 (341 – 471)	0.12	426 (372 – 482)	426 (363 – 478)	0.40
BODE Index	1 (0 – 2)	1 (0 – 2)	0.29	0 (0 – 1)	1 (0 – 1.5)	0.09
COPD Score (CAT)	13 (7–20)	13 (7–19.5)	0.45	12 (7–19)	13 (8–20)	0.18
SGRQ (Total)	31.5 (14.9 – 48.2)	31.2 (16.4 – 47.1)	0.81	26.2 (14.0 – 43.6)	31.8 (17.2 – 47.1)	
SGRQ (Symptoms)	45.3 (22.9 – 66.3)	48.8 (27.0 – 66.3)		45.2 (23.5 – 65.1)	53.6 (34 – 70)	
<i>Symptoms:</i>						
Wheezing, N (% positive)	741 (59)	788 (64)		389 (60)	116 (68)	0.07

* Mann-Whitney rank sum test for continuous variables, results as median (25–75% interquartile range); Chi-square for categorical variables, results as N (% positive response).

Comparison of exacerbations 1 (in the previous year) for subjects stratified by mean blood or sputum eosinophils (Eos; cutoff 200/ μ L or 1.25%, respectively). All values are percentage positive.

Table 5

Variable	BLOOD Eos<200	BLOOD Eos 200	P value*	SPUTUM Eos<1.25 %	SPUTUM Eos 1.25 %	P value*
N	1262	1237		656	171	
Definition of exacerbation:						
Total, N (%)	311 (25)	309 (25)	0.35	125 (19)	44 (26)	0.05
Healthcare Utilization, N (%)	294 (23)	291 (24)	0.36	125 (19)	43 (25)	0.07
Antibiotic treatment, N (%)	232 (18)	240 (19)	0.29	92 (14)	34 (20)	0.09
Corticosteroid treatment, N (%)	199 (16)	209 (17)	0.27	66 (10)	32 (19)	
Any drug-treatment, N (%)	265 (21)	273 (22)	0.29	105 (16)	39 (23)	
Severe, N (%)	137 (11)	162 (13)	0.15	52 (8)	22 (13)	

* Chi-square, results as % positive response.

Table 6

Spirometry and exacerbations for subjects in sputum (spt) versus blood (cbc) eosinophil (Eos) distribution stratified by quadrant (blood Eos cutpoint 200/ μ L; sputum Eos cutpoint 1.25%):

	Low spt Eos+ Low cbc Eos	Low spt Eos+ High cbc Eos	High spt Eos+ Low cbc Eos	High spt Eos+ High cbc Eos	P value*
N	355	290	50	116	
<i>Pre-bronchodilator:</i>					
FEV ₁ % pred	77 (62–91)	74 (57–89)	62 (51–81)	66 (50–79)	
FVC % pred	93 (81–101)	88 (77–100)	89 (76–99)	87 (76–96)	
ratio FEV ₁ /FVC	0.67 (0.58–0.74)	0.66 [‡] (0.57–0.73)	0.61 [‡] (0.47–0.66)	0.61 (0.52–0.69)	
<i>Post-bronchodilator:</i>					
FEV ₁ % pred	84 (70–97)	81 (66–95)	76 (63–87)	77 (63–90)	
FVC % pred	96 (86–106)	93 (84–104)	98 (88–104)	93 (85–104)	0.24
ratio FEV ₁ /FVC	0.69 (0.59–0.77)	0.67 [‡] (0.59–0.76)	0.63 [‡] (0.49–0.72)	0.64 (0.58–0.70)	
% reversibility	7.8 (3.6–15.3)	8.5 [‡] (4.0–16.6)	14.5 [‡] (6.8–22.6)	11.4 (5.8–20.5)	
<i>Definition of exacerbation:</i>					
Total N (%)	76 (21)	47 (16)	10 (20)	32 (28)	0.07
Requiring HCU N (%)	74 (21)	45 (16)	9 (18)	31 (27)	0.07
Antibiotics treatment N (%)	56 (16)	36 (12)	10 (20)	23 (20)	0.21
Corticosteroid treatment N (%)	37 (10)	25 (9)	6 (12)	24 (21)	
Any Drug treatment N (%)	61 (17)	38 (13)	10 (20)	27 (23)	0.08
Severe (ED or hospital) N (%)	35 (10)	15 (5)	3 (6)	17 (15)	

* Kruskal-Wallis for continuous variables, results as median (interquartile range); Chi-square for exacerbation categories, results as N (% positive response).

[‡]Indicates High sputum Eos+ Low cbc Eos quadrant was significantly different from Low spt Eos+ High cbc Eos quadrant by Dunn's method.